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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Miller et al.	Examiner: B. Celsa
Serial No.	:	09/181,108	
Cnfrm No.	:	9507	Art Unit: 1627
Filed	:	October 28, 1998	
For	:	COMBINATORIAL LIBRARIES	

DECLARATION OF BENJAMIN L. MILLER UNDER 37 CFR § 1.131

I, BENJAMIN L. MILLER, hereby declare:

1. I am an inventor of the above-identified application.

2. I am presenting this declaration to demonstrate that the claimed subject matter was invented, in the United States, prior to June 2, 1997. As demonstrated below, the claimed invention was reduced to practice prior to June 2, 1997.

3. Attached hereto as Exhibit A is a copy of a letter from me to Professor Allen J. Bard, the editor of the Journal of the American Chemical Society as of the time the letter was written. The letter was accompanied by a manuscript entitled: "Self-assembled Combinatorial Libraries Capable of Self-Selection and Amplification: Generation of Novel DNA-Binding Compounds" (copy attached as Exhibit B). Although the date of the letter has been redacted, the date of the letter is prior to June 2, 1997.

4. The above-identified manuscript attached as Exhibit B bears a date stamp from the Journal of the American Chemical Society, which indicates the date this manuscript was received. Although the date has been redacted, the date is prior to June 2, 1997. A code number, presumably applied by the Journal of the American Chemical Society, has also been redacted to the extent that it may reflect the exact date on which the manuscript was received.

5. The manuscript attached as Exhibit B describes the formation of a combinatorial library in an aqueous solution. The combinatorial library was formed by introducing into the aqueous solution: (i) divalent zinc ions in the form of $ZnCl_2$ and (ii) six salicylaldimines, identified as compounds 3-8 therein, with compounds 4 and 6 being prepared as racemic mixtures to afford eight distinct salicylaldimines. These salicylaldimines

are non-biopolymer ligands within the definition provided at page 6, lines 10-22 of the above-identified application. Each of the salicylaldimines, formed by the condensation of salicylaldehydes and commercially available amines, is characterized by the presence of (i) at least one functional group capable of bonding to the divalent zinc (e.g., see Figure 2 of Exhibit B) and (ii) a recognition element capable of binding a biological receptor (e.g., see discussion of selection against double-stranded oligo$(dA)_{12-18}$/poly(dT) tethered to cellulose resin at page 4 of Exhibit B).

6. The combinatorial library formed from the salicylaldimines and divalent zinc includes 36 different complexes, each formed of the divalent zinc and two salicylaldimines (see page 3 of Exhibit B). Thus, each different complex in the combinatorial library has a different combination of salicylaldimines reversibly bonded to the zinc ion.

7. Each of the two salicylaldimines is reversibly bonded to the zinc ion through the functional group capable of bonding thereto (see discussion of experimental design at page 2 and Figure 1 of Exhibit B). Due to the labile coordinate bond formed in each member of the combinatorial library, the combinatorial library will exist at equilibrium in the aqueous solution with the zinc ions and unbonded salicylaldimines (see discussion of experimental design at page 2 and Figure 1 of Exhibit B). By introducing the aqueous solution that contains the combinatorial library to a biological receptor bound to a solid support, those compounds with higher affinity to the receptor will bind to the receptor and cause an equilibrium shift in favor of the higher affinity compounds (see discussion of experimental design at page 2 and Figure 1 of Exhibit B, and discussion of results at page 4-5 and Figure 3 of Exhibit B).

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5-28-03

Benjamin L. Miller

UNIVERSITY OF
ROCHESTER

COLLEGE OF ARTS AND SCIENCE
DEPARTMENT OF CHEMISTRY

REDACTED

Professor Allen J. Bard, editor
Journal of the American Chemical Society
Department of Chemistry and Biochemistry
14th Street and Speedway
The University of Texas
Austin, Texas 78712-1167

Dear Professor Bard,

Enclosed please find four copies of our manuscript entitled "Self-Assembled Combinatorial Libraries Capable of Self-Selection and Amplification: Generation of Novel DNA-Binding Compounds" which I would like to have considered for publication as a Communication in the *Journal of the American Chemical Society*. The manuscript describes the first utilization of a solid-supported biopolymeric receptor (DNA) to drive the selection and amplification of the highest affinity coordination complex from a self-assembled, equilibrating combinatorial library of coordination complexes. This method should be of general utility for the identification and synthesis of small-molecule ligands for biopolymeric receptors.

As reviewers for the manuscript, I would suggest Professor Cynthia Burrows (Department of Chemistry, University of Utah, Salt Lake City, Utah 84112) and Professor John Griffin (Department of Chemistry, Stanford University, Stanford, California 94305), both of whom have studied the recognition of nucleic acids by transition metal complexes extensively.

Thank you for your consideration of our manuscript.

Sincerely,

Benjamin L. Miller
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SELF-ASSEMBLED COMBINATORIAL LIBRARIES CAPABLE OF SELF-SELECTION AND AMPLIFICATION: GENERATION OF NOVEL DNA-BINDING COMPOUNDS

Bryan Klekota, Mark H. Hammond, and Benjamin L. Miller*

Department of Chemistry, University of Rochester, Rochester, New York 14627

MUST BE CONDENSED BEFORE ACCEPTANCE

ABSTRACT: The selection and amplification of small molecules from equilibrating, self-assembled combinatorial libraries has great potential as a new strategy for ligand synthesis. We describe herein a general method which utilizes an immobilized receptor (i.e., an affinity reagent) to effect selection of high-affinity ligands. Using commercially available oligo(dT) double-stranded DNA-cellulose resin, a single coordination complex is identified from an equilibrating, self-assembled library of 36 bis(salicylaldiminato)-zinc coordination complexes. Subsequent solution-based binding constant measurements indicate that this complex binds to double-stranded oligo(dT) with an apparent K_D of 1.11 micromolar, while another (nonselected) complex is much lower in affinity.

The *in vitro* selection and amplification of oligonucleotides has been an extraordinarily successful method of generating biopolymers with specific receptor-binding or catalytic properties.[1] The development of analogous selection and amplification methods for small molecules, where the selection and amplification criteria are based strictly on differences in binding affinity to a receptor, would be of tremendous utility for ligand generation. However, while a few reports of kinetic[2] or thermodynamic[3,4] selection of nonbiopolymeric materials have recently appeared in the literature, this area remains largely unexplored.[5,6] We describe herein the first utilization of a biopolymeric receptor (DNA) to drive the selection and amplification of the highest affinity coordination complex from a self-assembled, equilibrating combinatorial library of coordination complexes. The experimental design is shown in schematic form in Figure 1. Introduction of a transition metal salt ("M") to a pool of compounds ("monomers") capable of forming coordination compounds with M should initiate a series of equilibria among various combinations of complexes. Assuming ligand exchange is reasonably fast, this equilibration will permit all combinatorially possible coordination complexes to be populated. By adding a receptor covalently linked to a solid support (an affinity resin), however, an additional equilibration would ensue involving the binding of coordination compounds to the receptor with varying degrees of affinity. Since some compounds might be expected to bind with higher affinity, those compounds would be depleted from the pool of equilibrating complexes. By simple mass balance rules, the equilibrium would then have to shift in favor of compounds that bind to the receptor, effectively utilizing the receptor as a catalyst for the synthesis of its own ligand.

While this concept could easily be applied to any type of biopolymer or other "receptor", double-stranded DNA was chosen as our initial target, for a number of reasons. First, the design and synthesis of sequence-selective DNA-binding agents is a problem of continuing fundamental and medical importance.[7] Second, the ladder-like structure of DNA is ideally suited to the application of a modular, self-assembly strategy. Finally, several oligonucleotide affinity resins are available commercially, allowing the experimental details of initial selection strategies to be determined without the need for costly oligonucleotide and affinity resin synthesis. As

"monomers", the readily available salicylaldimines **1** were chosen. Salicylaldimines are well known to form coordination complexes with a wide variety of transition metals,[8] and a substantial amount of structural information about these complexes is available. Because of its tetrahedral coordination geometry with most salicylaldimines[9] and compatibility with nucleic acids, divalent zinc was used as the metal for initial studies.

Six salicylaldimines (**3** - **8**) displaying a variety of sidechain functionality were synthesized by the condensation of salicylaldehyde and commercially available amines (Figure 2).[10] Given that compounds **4** and **6** were synthesized in racemic form, a combinatorial library formed from these six salicylaldimines could be expected to provide a maximum of 36 unique bis(salicylaldiminato)zinc complexes. These compounds were chosen in part because millimolar solutions of each could be prepared in buffered aqueous solution with 1% DMSO as cosolvent, a finding crucial to their successful utilization in a combinatorial library.

Although the ability of bis(salicylaldiminato)-zinc[11] and -nickel[12] complexes to self-assemble in halogenated solvents is well known, we felt it was important to verify that this could also occur in aqueous solution before attempting to construct a combinatorial library. Therefore, the dependence of the proton NMR spectrum for **7** on $ZnCl_2$ concentration in D_2O was examined. Characteristic of slow-exchange phenomena, substantial line broadening of aromatic, and, to a lesser extent, side-chain resonances, is observable on addition of substoichiometric quantities of $ZnCl_2$. In addition, chemical shifts change markedly. All peaks coalesce into a single set of sharply defined resonances as excess zinc is added. While these observations do not provide high-resolution structural information about these complexes,[13] they do indicate that complexation between the salicylaldimine and Zn^{2+} occurs, providing a single coordinated species.[14]

An obvious potential problem with the selection and amplification of a single compound from an equilibrating mixture of complexes is that of identifying the desired high-affinity species. First, if the selection is not complete for a single compound, the separation and identification of a potentially large number of complexes presents a significant analytical challenge. Furthermore, since reequilibration of complexes can occur once the affinity resin is removed, it might be

expected that binding information would be lost. However, we found that hydrolysis of mixtures of complexes with trifluoroacetic acid followed by neutralization and derivatization with excess 2-naphthoyl chloride provided a mixture of amide derivatives that was readily separable by standard reverse-phase HPLC. Application of this technique to the eluent from an affinity column would permit deconvolution of the library to provide information about the identity of binding complexes.

With a series of monomers, verification of self-assembly in aqueous solutions, and a separation method in hand, the experimental tools were in place to conduct an affinity selection and amplification experiment. A standard solution of 0.5 mM **3** - **8** (total concentration 3 mM in "monomer") was prepared in 10 mM Tris•HCl, 100 mM KCl, 1% DMSO, pH 7.5. To determine the effect of zinc on the observed selection, samples were prepared either with or without 26 mM $ZnCl_2$. Samples were allowed to incubate for one hour to allow an equilibrium mixture of complexes to form, and the latter were then individually added to affinity columns prepared from 30 mg of commercial poly(dT)-cellulose resin[15] preincubated with one A_{260} unit (approximately 7 nmol) of oligo $(dA)_{12\text{-}18}$[16] in 10 mM Tris•HCl, 100 mM KCl, pH 7.5 for formation of double-stranded DNA (giving an approximate, average complex:base pair ratio of 85:1). Following a two-hour incubation of the libraries on the resin, solutions were eluted, lyophilized, and derivatized as described above.

Figure 3 shows the results of affinity selection and amplification of our initial self-assembled combinatorial library. With data normalized to values observed in the absence of zinc, it is clear that monomers **4** and **6** are most strongly retained on the affinity column in the presence of zinc (i.e., less of these monomers is observed following elution and derivatization). The observation that twice as much of derivatized monomer **7** is eluted from the affinity column in the presence of zinc as in the absence of zinc suggests that this monomer participates in some kind of binding interaction with double-stranded oligo(dA)-oligo(dT) in the absence of zinc that is not available to either the **7-Zn-7** complex, or other complexes incorporating monomer **7**. These results would then require deconvolution of three possibilities for the strongest binding complex:

4-Zn-4, **6-Zn-6**, and **4-Zn-6** (including all stereoisomers) would all be candidates. However, results of control experiments rendered this question moot.

To ensure that selections observed were based on differential affinity for double-stranded DNA, rather than interactions with single-stranded DNA or with the underlying cellulose support, control experiments were conducted using single-stranded oligo(dT)-cellulose, and cellulose alone, under conditions identical to those for the double-stranded experiment. As shown in Figure 4, monomer **4** is retained on cellulose or oligo(dT)-cellulose to the same extent in the presence or absence of zinc, while **6** is more strongly retained on cellulose in the presence of zinc. This suggests that two out of the three possibilities for the strongest binding complex to double-stranded DNA (**6-Zn-6** and **6-Zn-4**) do not need to be considered, leaving **4-Zn-4** as likely the tightest DNA-binding complex. Other monomers are generally retained more strongly to single-stranded oligo(dT) cellulose in the absence of zinc; we are currently investigating why this is the case.

In order to verify that the results of the affinity selection experiment accurately reflected differences in binding affinities, UV binding titrations[17] were conducted for homodimeric complexes **5-Zn-5** (a presumed weak- or non-binding complex) and **4-Zn-4** (a presumed strong-binding complex). Since both of these complexes have strong UV absorbances in the range of 290-350 nm while poly(dA)-poly(dT) does not, the concentration of complex was held constant in the presence of varying concentrations of DNA in each case. Using commercially available poly(dA)•poly(dT), saturable binding was observable in each case. While any analysis of binding constants for these complexes is complicated by the homopolymeric, variable-length nature of the DNA used (providing multiple, identical binding sites), **4-Zn-4** bound to DNA with an apparent K_D of 1.11 micromolar, significantly stronger than **5-Zn-5** (27.8 micromolar). A simple affinity selection experiment conducted as described above, but utilizing only monomers **4** and **5**, further corroborates these observations. As shown in Figure 5, the ratio of derivatized **5**:**4** eluted following incubation on double-stranded oligo(dT)-cellulose doubles on addition of zinc.

In conclusion, we have demonstrated the first example of the selection and amplification of a DNA-binding compound from a self-assembled, equilibrating combinatorial library of

coordination complexes. This method should be generally applicable to the synthesis of compounds capable of binding sequence-selectively to DNA, RNA, and proteins, since the receptor itself determines the final composition of the bound compound. Experiments designed to test this hypothesis are in progress.

Supplementary data: Experimental and spectral data for the preparation of compounds **3**, **5**, **6**, and **8**; procedure and results for the ^{1}H NMR titration of $ZnCl_2$ into **5** (6 pages).

Acknowledgement: The authors thank Professor Eric T. Kool and Mr. Charles Karan for helpful discussions. We also thank Eric T. Kool for the use of his HPLC. Financial support from the University of Rochester is gratefully acknowledged. M. H. H. was supported in part by a National Science Foundation Research Experience for Undergraduates grant CHE-9322203.

References

(1) (a) Szostak, J. W. "*In vitro* genetics" *Trends Biochem. Sci.* **1992**, *17*, 89-93; (b) Tuerk, C.; Gold, L. "Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase" *Science* **1990**, *249*, 505-510.

(2) Amabilino, D. B.; Ashton, P. R.; Perez-Garcia, L; Stoddart, J. F. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2378-2380.

(3) Hasenknopf, B.; Lehn, J.-M.; Kneisel, B. O.; Baum, G.; Fenske, D. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1838-1840.

(4) Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37-44.

(5) A recent report has described a method for the selection of compounds capable of binding to guanidinium via a photoinduced isomerization (Eliseev, A. V.; Nelen, M. I. *J. Am. Chem. Soc.* **1997**,*119*, 1147-1148); however, selection is driven by an external energy source (light) in addition to differences in binding affinity.

(6) Huc and Lehn have provided an elegant demonstration of changes in product ratios obtained in imination reactions carried out on mixtures of amines and aldehydes in the presence of carbonic anhydrase: Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106-2110.

(7) For example, see Trauger, J. W.; Baird, E. E.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6160-6166 and references therein.

(8) Garnovskii, A. D.; Nivorozhkin, A. L.; Minkin, V. I. *Coord. Chem. Rev.* **1993**, *126*, 1-69.

(9) Dreher, M.; Elias, H.; Paulus, H. *Z. Naturforsch. B.* **1987**, *42*, 707-712.

(10) The synthesis and characterization of **3** and **5** have been previously described. Compound **3**: Inouye, S. *Chem. Pharm. Bull.* **1967**, *15*, 1540-1546; compound **5**: (a) Duran, M. L.; Romero, J.; Sousa, A. *Synth. React. Inorg. Met.-Org. Chem.* **1987**, *17*, 681-683. (b) Ramesh, K.; Mukherjee, R. *J. Chem. Soc. Dalton* **1992**, 83-86.

(11) Blackborow, J. R.; Karunaratne, S.; Lockhart, J. C.; Hill, M. N. S. *J. Chem. Res.* **1978**, 119.

(12) Schepartz, A.; McDevitt, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 5976-5977.

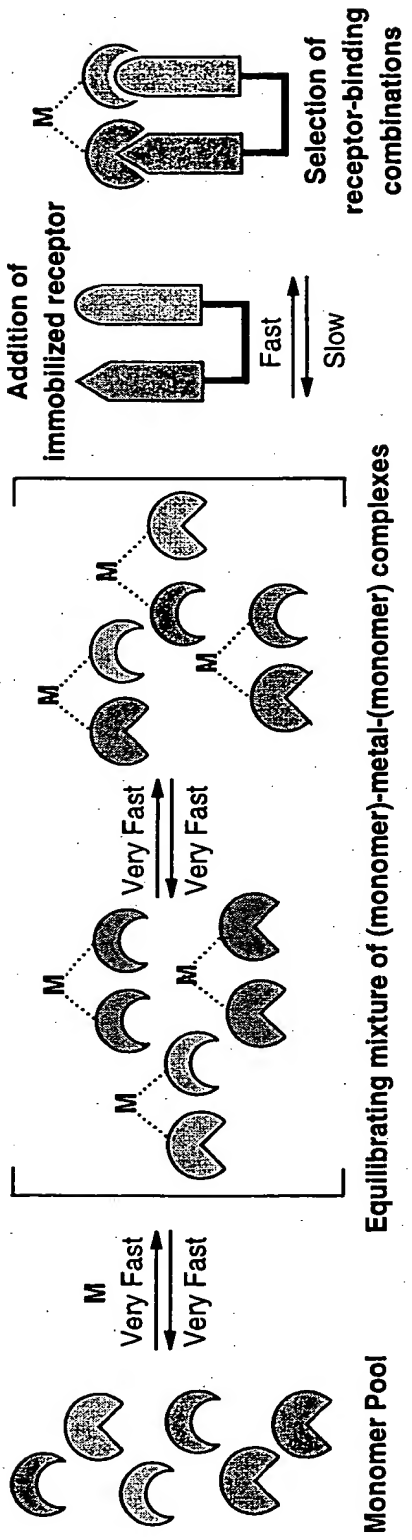
(13) It is conceivable that the structure in solution is an addition complex (containing two salicylaldimines coordinated to $ZnCl_2$) rather than a chelate: see Bottino, F. A.; Finocchiaro, P.; Libertini. E.; Mattern, G. *Zeitschrift für Kristallographie* **1989**, *187*, 71-77.

(14) We have also observed that the addition of $ZnCl_2$ to a solution of an amine and salicylaldehyde in water can induce imine formation followed by complexation (B.K. and B.L.M., unpublished data). This suggests that a three-stage equilibrium process could be constructed.

(15) Pharmacia Biotech

(16) Oligo $(dA)_{12-18}$ as obtained from Pharmacia Biotech is a mixture of homopolymeric, single-stranded DNA of length 12 to 18.

(17) Krugh, T. R. *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 1911-1914.


Monomer Pool
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Very Fast
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Equilibrating mixture of (monomer)-metal-(monomer) complexes
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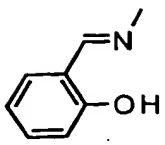
Figure 1



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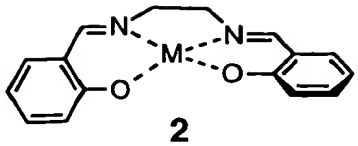

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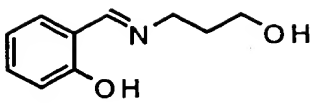

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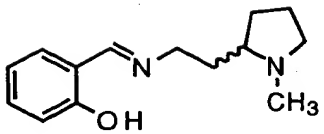

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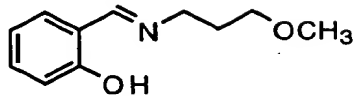

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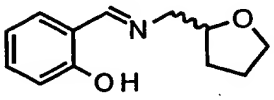

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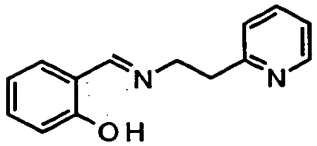

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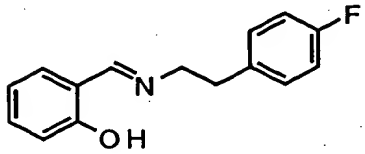

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Figure 2: Salicylaldimines synthesized for a trial Class I library.

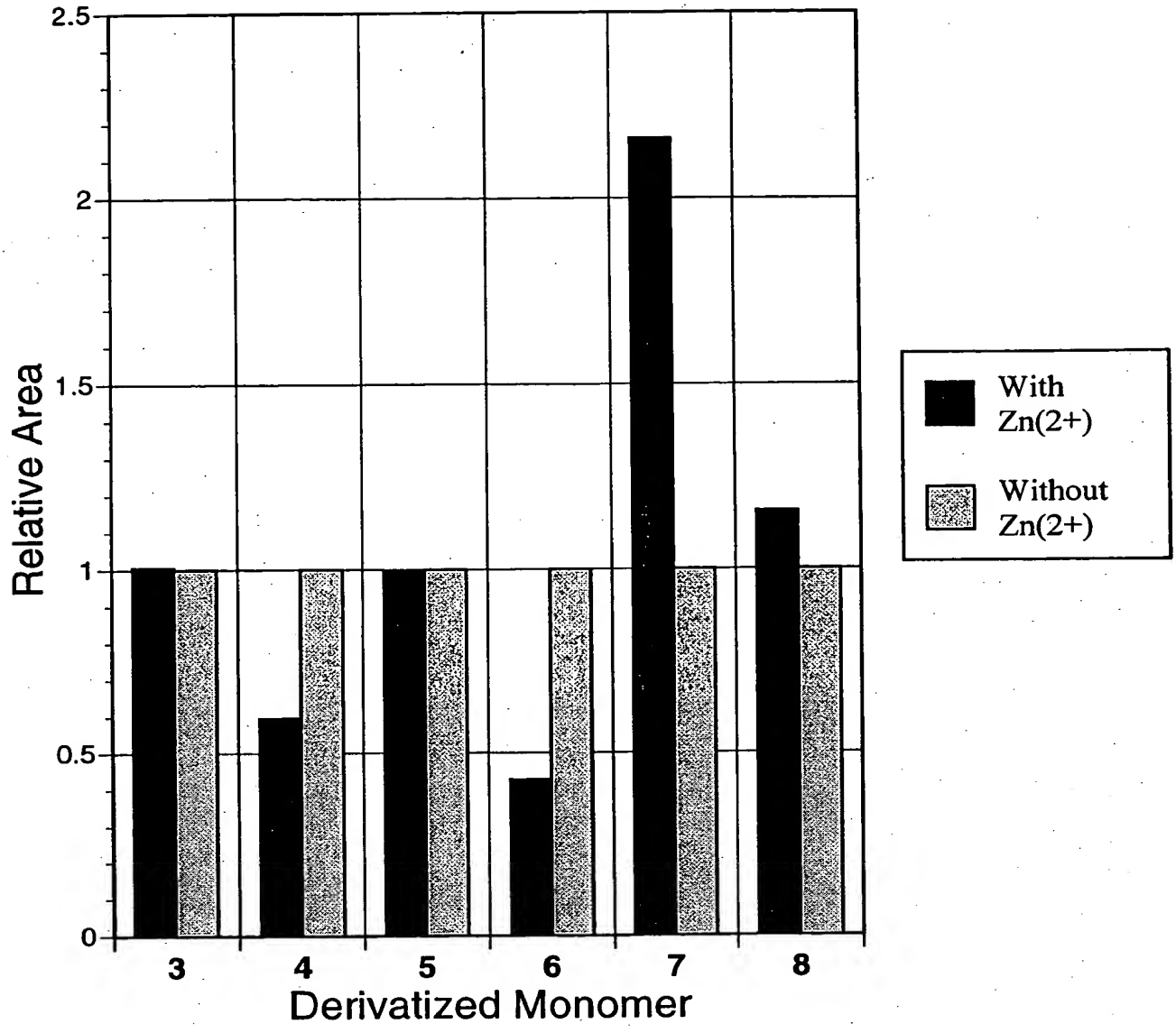


Figure 3: Results of affinity selection and amplification with double-stranded oligo(dT)-cellulose. Values are normalized to those observed in the absence of Zn^{2+}.

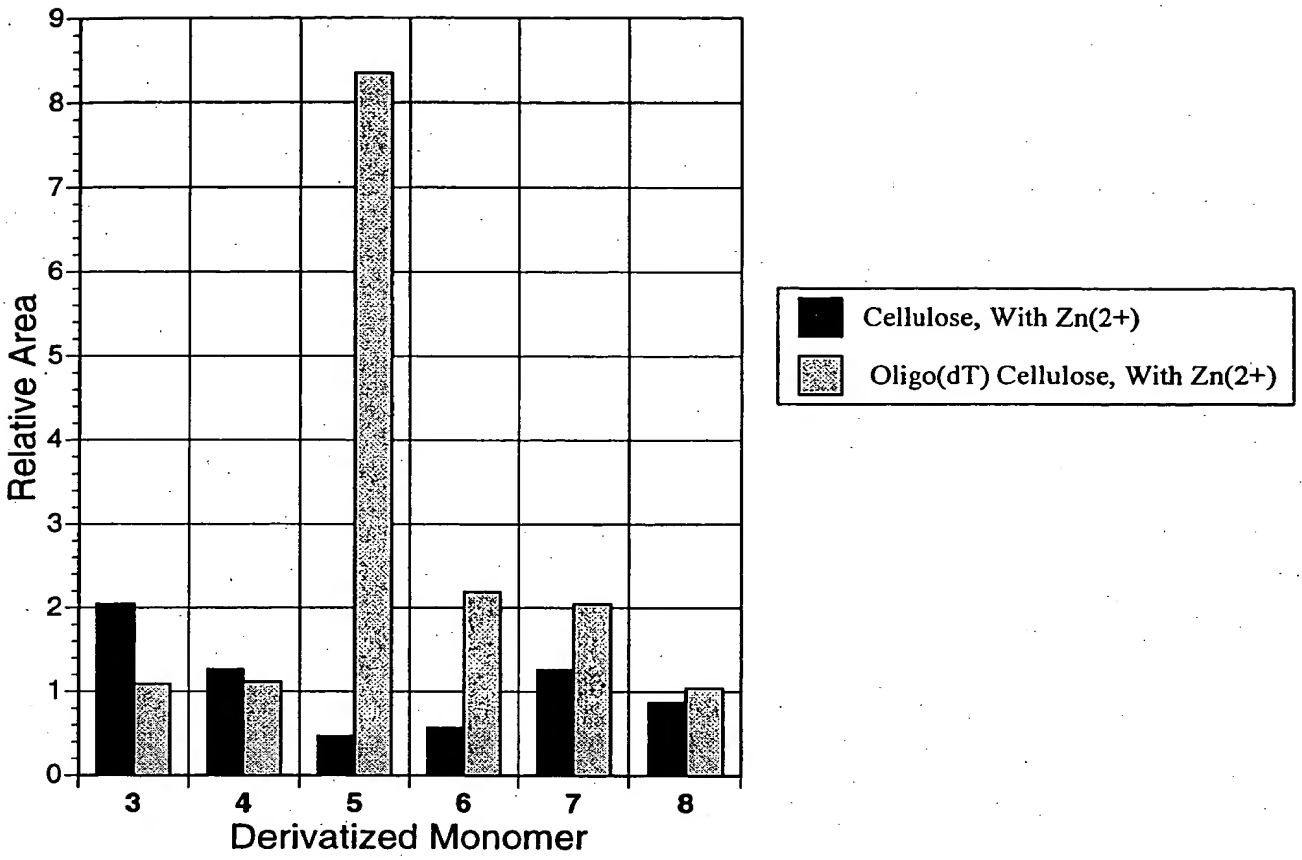


Figure 4: Results of control selections using cellulose or single-stranded olito(dT)-cellulose. Values are normalized to those observed for each control in the absence of Zn^{2+}.

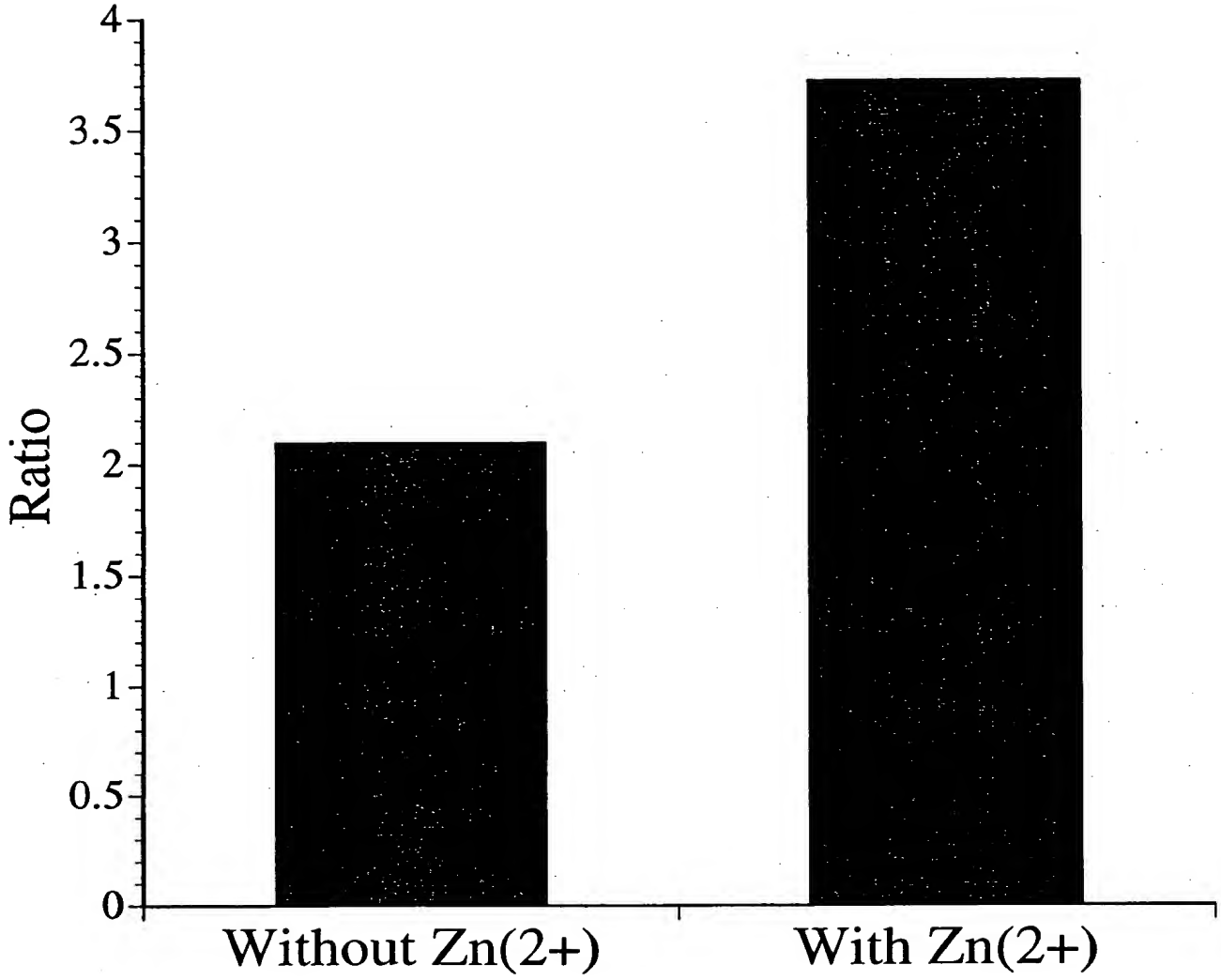
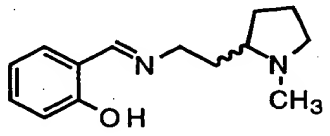


Figure 5: Ratio of derivatized **5**:**4** eluted from double-stranded oligo(dT) affinity resin in the presence and absence of zinc.

SUPPLEMENTARY MATERIAL

General Experimental Procedures: All solvents, salicylaldehyde, and amines were obtained from Aldrich Chemical Company and were used without further purification. NMR spectra were recorded on a Nicolet/GE QE-300 FT spectrometer operating at 300 (^{1}H) or 75 (^{13}C) MHz. IR spectra were recorded using a Perkin-Elmer model 1600 FTIR. High-resolution mass spectra (HRMS) analyses were carried out by the UC-Riverside Mass Spectrometry Facility, Riverside, California.

Preparation of **4**: To a solution of salicylaldehyde (3.0 mmol) and 2-(2-aminoethyl)-1-methyl pyrrolidine (3.0 mmol) was added in dichloromethane was added an excess (1 ml, or approximately 2.6 equivalents) triethylamine. Reaction was allowed to proceed with stirring at room temperature for two hours. Solvent and triethylamine were then removed *in vacuo* to provide essentially pure **4** in quantitative yield.



4

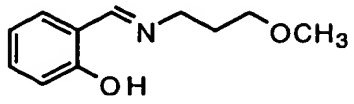
^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.15 (s, 1H), 8.39 (s, 1H), 7.3 (m, 2H), 6.9 (m, 2H), 3.62 (m, 2H), 3.1 (m, 1H), 2.34 (s, 3H), 2.1 (m, 4H), 1.7 (m, 4H).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.6, 161.2, 132.1, 131, 118.8, 118.4, 117, 64.2, 57.2, 57.1, 40.5, 35.1, 30.8, 22

IR (thin film): 3788.7, 3695.5, 3661.2, 3636.2, 2941.2, 2840.0, 2776.5, 2666.1, 2360.0, 2341.5, 1725.3, 1709.5, 1688.4, 1664.0, 1632.4, 1581.0, 1549.5, 1497.1, 1460.4, 1415.5, 1370.4, 1344.2, 1280.3, 1212.4, 1150.9, 1117.2, 1031.6, 970.1, 891.3, 847.6, 755.8, 736.2, 668.0

HRMS: calc. 233.1528; obs. 233.1647

Preparation of **5**: As with **4**, from 3-methoxypropylamine:



5

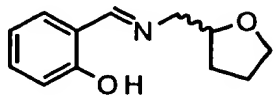
^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.57 (s, 1H), 8.19 (s, 1H), 6.55 (m, 2H), 6.4 (d, 1H, J=9 Hz), 6.38 (t, 1H, J=9 Hz), 2.9 (t, 2H, J=7.5 Hz), 2.8 (t, 2H, J=7.5 Hz), 2.78 (s, 3H), 2.0 (q, 2H, J=7.5 Hz).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 165, 161, 135, 133, 119, 118, 116, 70, 59, 56, 32.

IR (thin film): 3845.6, 3788.5, 3695.5, 3681.7, 1660.9, 3638.0, 2924.1, 2870.0, 1725.3, 1709.8, 1689.2, 1657.4, 1632.5, 1581.3, 1549.6, 1529.2, 1497.5, 1460.8, 1414.6, 1384.6, 1337.6, 1279.6, 1202.0, 1151.0, 1118.1, 1031.3, 974.1, 849.0, 756.4, 736.4, 640.1

HRMS: calc. 194.1055; obs. 194.1187

Preparation of **6**: As with **4**, from 2-aminomethylfuran:



6

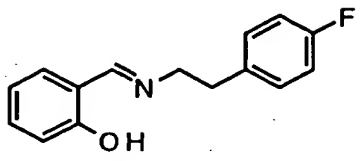
^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.19 (s, 1H), 8.40 (s, 1H), 7.3 (m, 2H), 6.9 (m, 2H), 4.2 (m, 1H), 3.8 (m, 4H), 1.95 (m, 4H).

^{13}C NMR ($CDCl_3$, 75 MHz): δ166.31, 161.70, 132.64, 131.69, 119.28, 118.89, 117.44, 78.61, 68.86, 63.91, 29.59, 26.29.

IR (thin film): 3947.8, 3256.7, 3055.7, 2871.7, 2733.4, 2661.7, 2061.3, 1945.4, 1912.4, 1790.0, 1633.1, 1580.7, 1496.2, 1461.8, 1416.4, 1365.4, 1315.8, 1281.5, 1246.9, 1209.6, 1151.2, 1116.1, 1074.6, 1046.3, 1026.7, 996.6, 966.4, 922.1.

HRMS: calc. 206.1182; obs. 206.1185

Preparation of **8**: As with **4**, from 4-fluorophenethylamine:



8

^{1}H NMR ($CDCl_3$, 300 MHz): δ 13.39 (s, 1H) 8.1 (s, 1H), 7.35 (m, 1H), 7.2 (m, 2H), 7.0 (m, 2H), 6.9 (m, 2H), 3.8 (t, 2H, J=7.5 Hz), 3.0 (t, 2H, J=7.5 Hz).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 168, 164, 161, 159, 135, 133, 132, 130, 118, 117.5, 115, 114.5, 68, 61, 36.5.

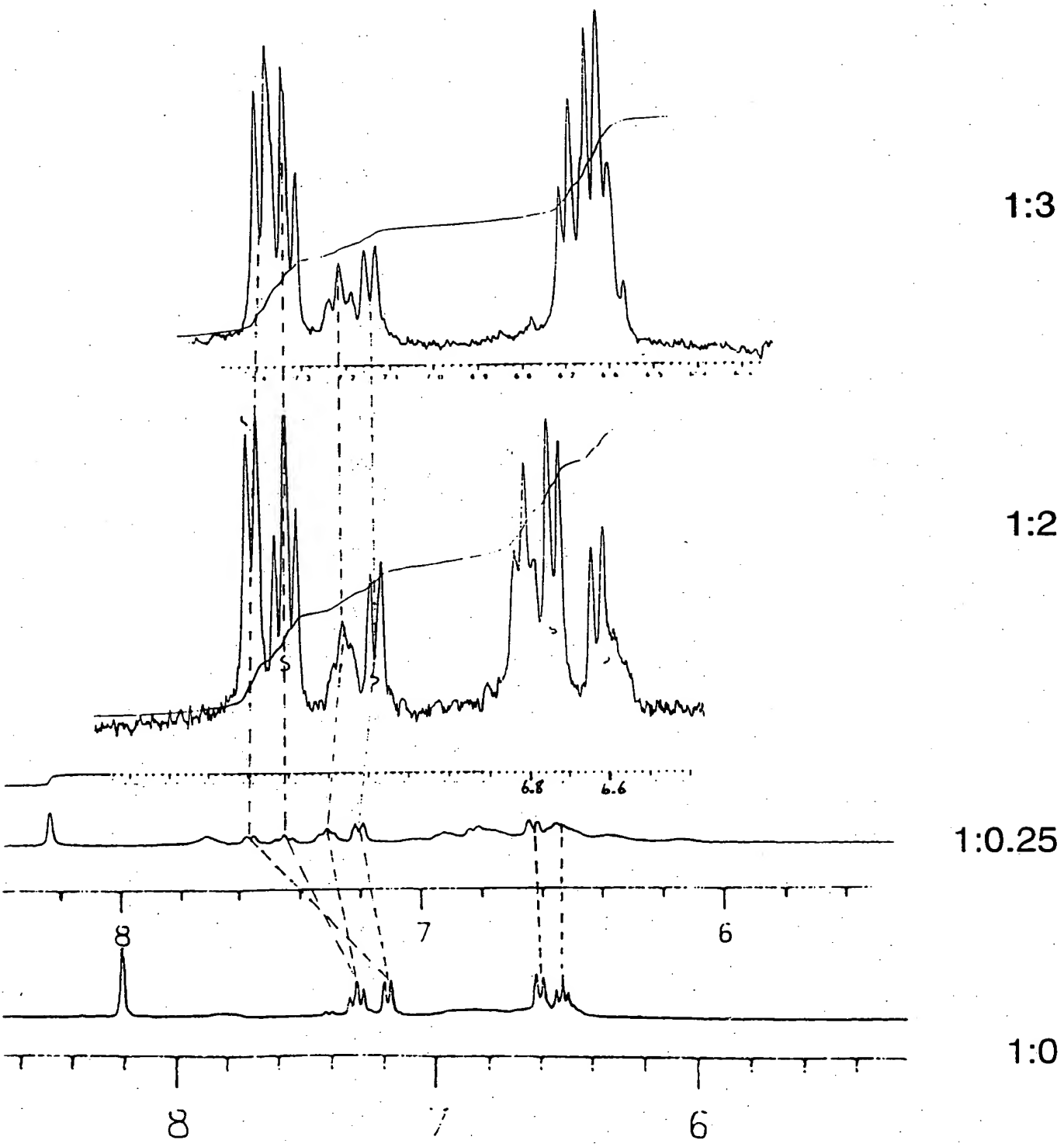
IR (thin film): 3044.6, 2926.6, 2852.4, 2732.1, 2662.9, 1887.1, 1633.2, 1607.6, 1580.0, 1509.6, 1460.7, 1415.6, 1376.4, 1342.1, 1279.9, 1221.2, 1156.8, 1116.8, 1098.2, 1073.7, 1053.0, 1016.1, 9687.6, 894.1, 870.1, 824.5, 778.1, 757.0, 736.3, 724.3, 705.5, 653.1, 640.8, 573.8, 542.5, 509.5, 481.9, 467.6.

HRMS:calc. 244.1011; obs 244.1129.

NMR Titration of $ZnCl_2$ into **5**:

A 97 mM solution of **5** in deuterated buffer (PBS, pH 7.4) was prepared, and the ^1H NMR spectrum measured at 300 MHz, 23 °C. Aliquots of a 1M solution of $ZnCl_2$ were then added, to permit measurement of the NMR spectrum at 1:0.25, 1:2, and 1:3 molar ratios of **5**:Zn^{2+}. Plots of the observed NMR spectra are shown on the following pages.

5:$ZnCl_2$



5:$ZnCl_2$